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AD835843

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AD835643

TRANSLATION NO. 1663

[redacted]

DATE: 15 April 1968

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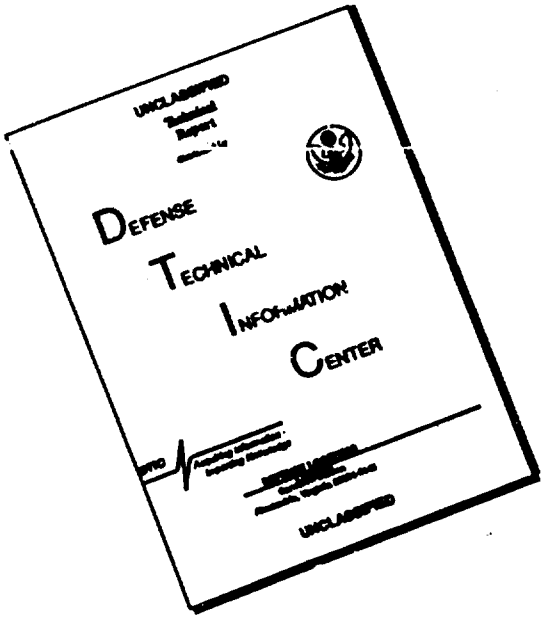

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HIBERNATION OF WHITE RICE LEAF WILT BACTERIA IN SOIL

..KIMOTO, Tetsu

Nogyo Gyobi Engei
(Agriculture and
Horticulture), No. 31(10),
1965, pp. 1413-14

The pathogenes of white rice leaf wilt are considered to be able to hibernate in two different forms. [9] One is the dried type believed to be found when hibernating in diseased leaves or seed rice stored indoors. The other type is the bacteria in the ordinary proliferation state in which it was formerly believed to hibernate even in the soils of the rice field where the disease normally is prevalent. Experimental proof of the former has already been reported [6, 8] while the latter has not exceeded the area of conjecture based on indoor laboratory tests with no direct evidence being observed. The method of detecting specific bacteria by means of bacteriophage [6, 7] is a very sensitive and extremely convenient method for empirically proving the above facts. The author employed the OP_1 phage [7] (one system of white rice leaf wilt pathogenic bacteriophage) and has already been able to establish several new facts. Here one new fact has been established regarding the existence of the bacteria in the soil, the establishment of which fact was one of the greatest difficulties with respect to the hibernation of the pathogenes of the "white wilting of rice leaves" disease. It appears that the pathogenes of white wilt of rice leaves hibernate around the roots of the sayanukagusa [a type of grass] (also possibly in wheat, rape-seed, broad bean, suzumeno teppo [sparrow's rifle]). It is a fact that the pathogenes are abundant around the root or in the ground around the sayanukagusa growing in marshes already in the last part of April. Below I shall describe briefly the experimental method and results.

1. Materials and Method

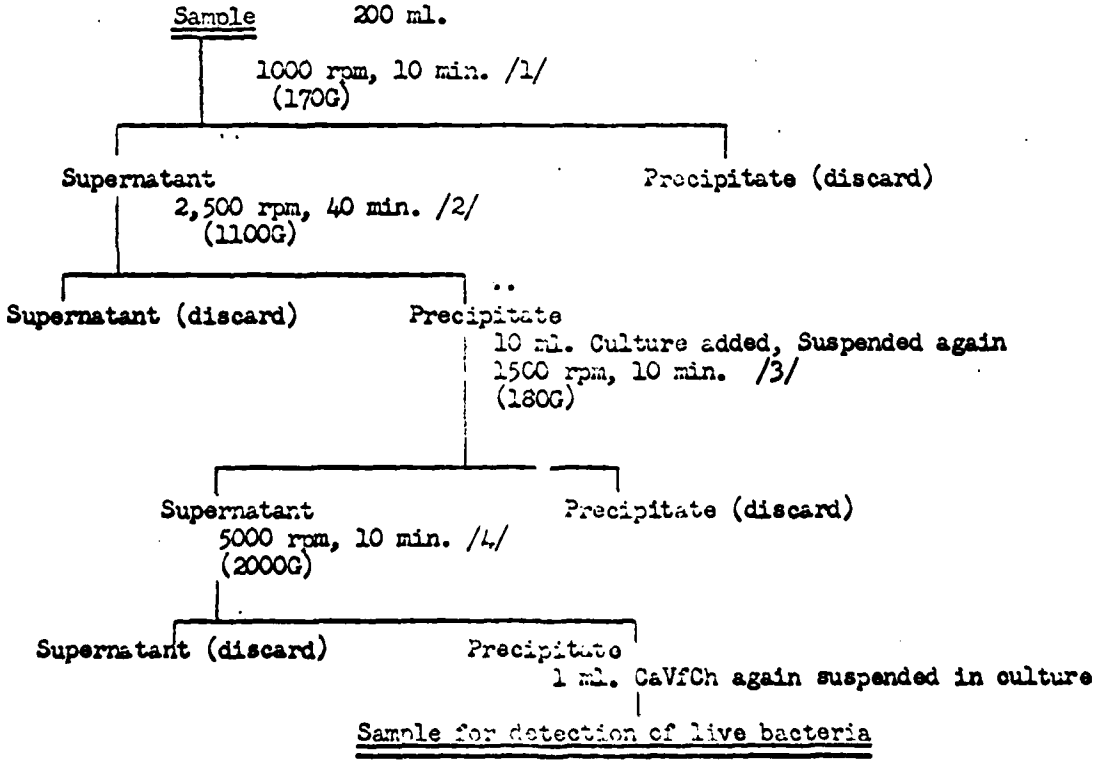
The items tested were crops cultivated in the winter in the vicinity of the fields[5] of Taramachi, Fujisawa-gun, Saga Prefecture. The roots of rape wheat, broad bean and suzume no teppo and the soil attached to them, the diseased dead leaves, roots and attached soil of the sayanukagusa which

grows in the marshes and is regarded as the source of the disease in the area, and the marsh water were collected and tested. Twenty grams of dead, diseased savanukamusa leaves were crushed in a mortar. The roots of the various plants were shaken in order to remove the soil, many roots were placed in a flask, 250 ml of antibacterial solution was added and the mixture was stirred adequately. After 30 minutes the supernatant liquid (containing, of course, a large amount of soil) was separated by centrifuge, the living bacteria fraction was collected and used to detect the pathogenes of white wilting of rice leaves, the centrifuge operation is shown in figure 1.

The objective of this centrifugal "fractioning" operation is to remove the soil which interferes with the phage test by means of one low speed and one high speed cycle in order to obtain only the bacteria.

The phage method of detecting white rice wilt has already been described in a previous report. Here an especially highly sensitive method of analysis [7] was also used.

Figure 1: Method of Preparing Samples for Detection of Live Bacteria in Soil.



Notes: /1/ /2/ Used a large Kubota Centrifugal separator
/3/ /4/ Used a high speed Kubota Centrifugal separator

2. Results

The samples were collected from ordinary growth areas and tested on March 12,, April 30 and May 17. The results are shown in Table 1, 2, 3.

Table 1: Existence of bacteria of white rice wilt near and around plant roots (Sample collected March 12).

	Check (0.01 ml)	Test (0.01 ml)	Positive/ Negative
Sayanukagusa (Root)	11, 7	34, 28	±
Sayanukagusa (Dead, Diseased leaf)	17, 27	14, 10	-
Rape (Root)	13, 17	37, 32	±
Wheat (Root)	29, 32	31, 32	±

Note: Numbers are number of bacteria-containing spots which appeared on two sheets of share /Note: This word is borrowed from English or some other European language./

Table 2: Existence of bacteria of white rice wilt near and around plant roots (sample collected April 30)

	Check (0.05 ml)	Test (0.05 ml)	Positive Negative
Broad bean (root)	250, 190	260, 280	±
Rape (root)	198, 152	290, 230	±
Suzumenoteppo (root)	165, 180	280, 310	±
Wheat (root)	211, 212	255, 260	±
Sayanukagusa (root)	220, 210	N, N	+
Marsh water	220, 236	350, 420	±

Note: Indicates instances in which bacterial spots were too many to count believed to be more than 7,000.

Table 3: Existence of bacteria of white rice wilt near and around plant roots (sample collected May 17)

	Check (0.01 ml)	Test (0.01 ml)	Positive Negative
Wheat (root) (a)	25, 26	2, 2	-
Wheat (root) (b)	10, 11	21, 21	±
Sayanukagusa (Root) (a)	135, 150	N, N	+
Sayanukagusa (Root) (b)	19, 13	390, 360	+
Rape (root) (b)	280, 230	245, 250	±
Marsh water (b)	140, 143	350, 330	+

Note: (a) = Samples collected in Takagisemachi in Saga City
(b) = Samples collected in Taramachi Fujitsu-gun, Saga Prefecture.

3. Considerations

The method of concentrating the bacteria in soil by centrifugal separation has as its purpose to remove soil particles which pose the problem of adherence to the phage in carrying out the detection of like bacteria by the bacteriophage method and to obtain only the bacteria fractions. Since it is naturally assumed that soil particles have bacteria adhering to them, considerable loss is inevitable. However, if many samples are taken employing a large centrifuge it is possible to reduce this danger when preparing samples for live bacteria tests. If the method of analysis of bacteria by means of bacteriophage[7] is applied to the separated samples, it should definitely be possible to establish whether the "target" bacteria exist.

Table 1, 2 and 3 give the results of checking on the existence of "white wilting of rice leaves" bacteria around the roots of various plants collected from usual areas of prevalence on 12 March, 30 April and 17 May. According to this method, their existence in winter is suspected. This is especially apparent around the roots of sayanukagusa where the bacteria are growing especially profusely already in the last part of April. The observation of primary outbreak of the disease in sayanukagusa in June also lends support to the hypothesis of this being a hibernation area.

As described above, the pathogenes of white rice leaf wilt can hibernate in a dry state in diseased leaves and seed rice stored indoors. However, it is clear that the bacteria in such a dry state gradually die

off when they become included in ordinary field soil in the winter and when they sustain water at low temperatures [9]. Moreover, areas of hibernation in a state of proliferation were previously suspected and the test herein have confirmed those suspicions.

Here I have explained in brief the hibernation of pathogenic bacteria of white rice leaf wilting disease. It would be safe to conclude that the bacteria have a dry condition in seed rice in individual diseased plants stored indoors and also hibernate in the profuse state around the roots of plants, especially savanukamusa.

I wish to express my gratitude to professor Yoshii Hajime for his guidance on details and to professor Minakami Takeyuki of Saga University and technician Seki Masao of the Saga Agricultural Test Station for their help in collection.

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